Environmental Health Perspectives
Vol. 27, pp. 37–43, 1978

Chromosome Aberrations in Plants as a Monitoring System

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The potential of higher plants as a first-tier assay system for detecting chemical mutagens is evaluated. The use of plant tissue (primarily root tips and pollen mother cells) for studying the induction of chromosomal aberrations is one of the oldest, simplest, most reliable, and inexpensive methods available. Specific types of abnormalities have been induced by different classes of pesticides. Chromosome clumping, contraction, stickiness, paling, fragmentation, dissolution, chromosome and chromatid bridges, C-mitosis, and endoploidy have been reported in the literature. Examples of cytogenetic studies with pesticides demonstrating the usefulness of higher plants as a monitoring system are reviewed. Pesticides which cause chromosome aberrations in plant cells also produce chromosome aberrations in cultured animal cells. Frequently, the aberrations are identical. For example, studies have shown that compounds which have a C-mitotic effect on plant cells have the same effect on animal cells. It is recommended that plant systems be accepted as a first-tier assay system for the detection of possible genetic damage by environmental chemicals.

Introduction

Chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological and taxonomical changes, fertility-sterility relationships, mutations, and other characteristics. The first observation of a correlation between reduction in fertility and cytological abnormalities as a result of pesticide treatment dates back to 1931, when Kostoff observed seed set of tobacco plants to be greatly reduced after the plants had been fumigated with nicotine sulfate. In an examination of meiosis, Kostoff (*1*) found many chromosome irregularities which he considered to be the cause of the partial sterility of the plants. Subsequent studies with many mutagenic chemicals have shown that plant chromosomes exhibit many different types of aberrations some of which are specific for different chemicals or classes of chemicals.

In the present paper, some aspects of the relevance and reliability of chromosome aberrations in plants as a method for the detection of possible genetic damage by environmental agents are discussed. Examples of the effects of treatment with pesticides (that is, herbicides, insecticides, and fungicides) are presented. In general, chromosome aberrations can provide both qualitative and quantitative data on the effects of exposure to a mutagen, and examples are given. In addition, parallels between chromosome aberrations caused by the same pesticides in plant and mammalian systems were to demonstrate that plant systems would serve as an excellent first-tier bioassay system.

Monitoring for Chromosome Abnormalities

One of the principal objectives of using chromosomes as a monitoring system is to determine whether or not a particular chemical is a clastogen (that is, capable of breaking chromosomes). If the chemical is a clastogen, then this would permit exchanges with subsequent cytological or genetic damage. At the same time it has been recognized that turbagens [chemicals which cause mitotic disturbances; a term proposed by Brøgger (*2*)], while not necessarily affecting DNA directly, may result in chromosome segregation errors, and therefore, should not be considered genetically insignificant.

Cytological aberrations in plants serve as an excellent monitoring system for the detection of environmental chemicals that may pose a genetic hazard. The plant systems which have proven most

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useful for this purpose have been reviewed recently by Nilan and Vig (*3*).

Chromosome aberrations may be detected in both mitotic and meiotic divisions. Structural rearrangements, which are most evident at metaphase and anaphase, are identical in somatic and gametic cells. Analysis of somatic chromosome aberrations may be carried out by using actively dividing root tip, stem apex, or pollen tube cells. Meiotic chromosome studies are usually carried out using pollen mother cells. In contrast, micronuclei are best detected at the quartet stage. Micronuclei, which vary in number and size, generally result from fragments or lagging chromosomes.

Types of Chromosome Abnormalities Induced by Pesticides

Nearly all of the common types of known cytological aberrations have been reported in plants following treatment with pesticides. The most frequently reported types will be discussed in the following sections.

Colchicine Mitosis

Levan (*4*) described colchicine mitosis as an inactivation of the spindle followed by a random scattering of the chromosomes over the cell. Delayed centromere division may result in the chromosomes assuming the characteristic C-pairs configuration in which sister chromatids, while remaining attached at the centromere, no longer remain adjacent to one another. C-mitotic compounds which interfere with the division of the cell nucleus are also classified as spindle poisons, mitotic poisons or antimitotic compounds.

There are a number of pesticides which are typical C-mitotic agents (Table 1). The carbamates, including barban (*5*), benomyl (*6*), carbaryl (*7*), chlorpropham (*5, 8*), propham (*5, 9, 10*) and diallate (*11, 12*); also BHC (*13, 14*) and the mercurials (*15, 16, 17*) are extremely active C-mitotic chemicals. The carbamates have been so effective as C-mitotic chemicals that several have been recommended for the artificial induction of polyploidy (*18*). Polyploidy has been induced as high as 16-ploid with the carbamate propham (*10*).

That plant systems are sensitive indicators of cytological aberrations is clear from studies by Fernandez-Gomez (*19*) and Fernandez-Gomez et al. (*14*) on the C-mitotic effect of the four isomers (α, β, γ, δ) of hexachlorocyclohexane. They found that the β isomer had no C-mitotic effect, the α isomer produced partial C-mitosis, while the γ and δ isomers resulted in complete C-mitosis. C-mitotic behavior in plants is a function of chemical concentration. If the C-mitotic agent is applied in too high a concentration, as with other chemicals (Fig. 1), mitosis may be completely arrested. Dilute solutions will induce partial or incomplete C-mitosis resulting in multipolar spindles, aneuploid nuclei, and micronuclei in addition to cells exhibiting normal mitoses. Thus plants are reliable indicators of C-mitotic behavior; and, as will be mentioned later, plant cells exhibit the same C-mitotic behavior as animal cells.

Table 1. Pesticides with C-mitotic activity.

Pesticides	
Mercury compounds	*Allium, Vicia, Crepis,* human leukocytes
Carbamates	*Allium, Avena, Secale, Hordeum, Zea, Tradescantia, Pisum*
Dieldrin	*Crepis*
Nitralin	*Allium, Tradescantia*
Hexachlorocyclohexane	33 species in 28 references; *Allium* 12, *Vicia, Zea, Triticum, Hordeum, Secale*

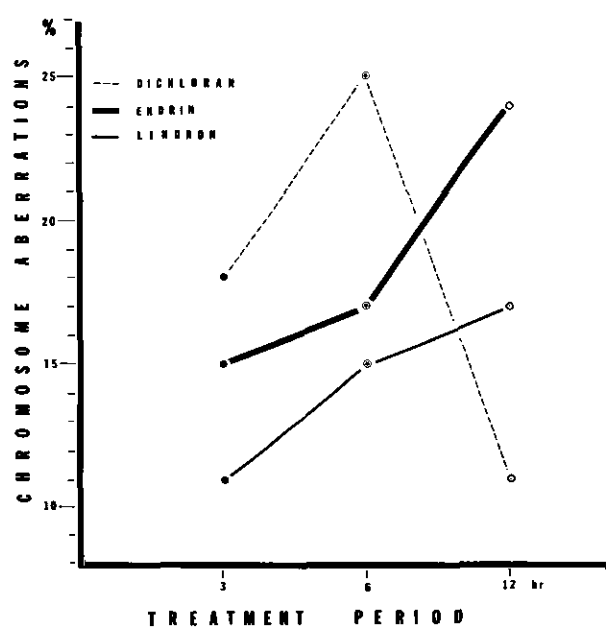

FIGURE 1. Chromosome aberrations in root tips of *Vicia faba* from treatments with three pesticides showing effect of toxicity from concentration and duration of treatment (dichloran and endrin, mean percentage of three concentrations, 100, 200 and 300 ppm; linuron, 200, 400 and 600 ppm). Data from Wuu and Grant (*55*).

Binucleate, Multinucleate, and Polyploid Cells

Binucleate cells arise as a consequence of the inhibition of cell plate formation. These form a distinct sub-population of easily detected cells. Failure of cell plate formation in already binucleate cells may give rise to the multinucleate condition. Mitotic irregularities, such as incompleted anaphases or unequal distribution of the chromosomes to the daughter cells can result in aneuploid or even euploid cells.

Several pesticides are known to induce the binucleate and multinucleate conditions including bromacil (*20*), carbaryl (*7*), dinoseb (*8*), hexachlorocyclohexane (*21–23*), nitralin (*24*), and propham (*9, 25, 26*). Tri- and tetrapolar anaphases have also been reported following treatments with certain of the preceding pesticides.

The induction of euploid cells has been reported after treatment with hexachlorocyclohexane (*13, 27–29*), and of aneuploid cells from chloranil treatment of root tips of *Vicia faba* (*30*) and atrazine treatment of *Sorghum* (*31*).

Endoreduplication, in which chromosome duplication occurs without nuclear division has been reported after 2,4-D treatment (*32*).

Chromosome Condensation and Contraction

Chromosome condensation or contraction is the shortening and thickening of the chromosomes brought about by changes in chromosome coiling following chemical treatment during mitosis and meiosis. Chromosome contraction has been observed following treatment of *Tradescantia* root tips with mercury compounds (*17*) and some carbamates (*18*).

Chromosome Stickiness and Clumping

Klasterska et al. (*33*) and McGill et al. (*34*) suggested that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and chromosomes. As a result there is an intermingling of the fibers, and the chromosomes become attached to each other by means of subchromatid bridges.

Chromosome stickiness and clumping have been reported following treatment with a number of pesticides including asulam (*35*), carbaryl (*7, 36, 37*), 2,4-D and 2,4,5-T (*38–40*); demeton (*41*), isodrin (*42*), mercurials (*17*), pentachlorophenol (*43*), and phosdrin (*44*).

Chromosome Haziness or Paling

Haziness or paling of chromosomes probably results from a partial despiralization of the chromosome. Haziness or despiralization has been reported after treatment with the carbamates, chlorpropham and propham (*45*) and nitralin (*24*).

Interchromatid Connections

Chromatin fibers which join two sister chromatids at metaphase and presumably hold the chromatids together until anaphase have been termed interchromatid connections (*46*). Such interchromatid connections have been observed after treatment of *Tradescantia* and *Vicia faba* root tip cells with a mercurial fungicide (*17*).

Chromosome Dissolution

Chromosome dissolution refers to a complete breakdown in chromosome structure resulting in the formation of long, thin chromatin threads which possibly arise from an almost complete despiralization of the chromosome. Chromosome dissolution has been observed in barley cells after seed treatment with monuron. The long chromatin threads form bridges between aggregations of chromosomal material (*47*).

Chromosome Fragmentation

Chromosome fragmentation results from multiple breaks of the chromosome in which there is a loss of chromosome integrity. Fragmentation can range from partial to total disintegration of the chromosome (the latter is termed chromosome pulverization). Chromosome fragmentation in plant cells has been reported only rarely after treatment with pesticides.

Amer and Ali (*43*) reported that pentachlorophenol induced fragmentation of both mitotic and meiotic chromosomes of *Vicia faba*. Other pesticides which have been reported to induce fragmentation include ferbam in *Allium cepa* (*48*), linuron in *Hordeum* (*49, 50*) and simazine in *Vicia cracca* (*51*).

Intensely stained interphase micronuclei, termed chromatin bodies, which result from chromosome fragmentation or aberrations in the previous mitotic division, have been observed in root tip cells of *Vicia faba* from treatment with amitrole and *Allium cepa* after 2,4-D treatment (*52*).

Chromosome Breakage and Exchange

The most common abnormalities recorded in these categories are (a) chromosome and chromatid breaks, (b) acentric fragments, (c) chromatid and subchromatid exchanges, chromatid gaps (achromatic lesions), heterochromatic regions and sister chromatid exchanges at metaphase, (d) chromatid and chromosome bridges and side-arm bridges and fragments at anaphase. A very detailed classification system has been proposed by Savage (*53*).

Chromosome breaks, fragments, chromatid exchanges, and dicentric chromosomes are generally considered unstable aberrations; deletions, inversions, duplications, and translocations are considered stable aberrations. Chromosome breakage is now generally considered to involve the DNA molecule responsible for the linear continuity of the chromosome. Such aberrations are the result of unfinished repair or misrepair of DNA (*54*).

The specific type of aberration induced is a function of the time at which the interphase nucleus is exposed to a clastogen. Exposure in the G_1 phase of the mitotic cycle results in damage to the entire chromosome while treatment in the S or G_2 phase results in damage to individual chromatids. Following treatment in the S phase, the typical aberrations encountered are chromatid breaks and chromatid interchanges. Exposure in the G_2 phase gives rise mainly to chromatid breaks and chromatid gaps. However, it should be noted that cells undergoing additional mitoses usually contain aberrations of the chromosomal type.

Many pesticides are clastogens, producing chromosome breaks which may give rise to anaphase bridges and fragements (*15, 47, 50, 51, 55, 56*). Since pesticides are not a homogeneous class of chemicals, their mode of action may be very different. For example, Ehrenberg (personal communication) has stated that the physiological action of phenoxy acids in higher plants should be considered since such compounds might secondarily lead to disturbances, including heritable changes, and therefore, the mechanism by which chromosomal aberrations are produced with such compounds should be clarified.

Some pesticides have been shown to consistently induce aberrations in specific regions of the chromosome in contrast to the random distribution observed after irradiation. For example, the growth retarding chemical maleic hydrazide induces chromosome breakage largely in heterochromatic regions (*57*). Similarly, Nicoloff and Gecheff (*58*) have shown that in barley seeds, following treatment with ethylenimine, the greatest portion of aberrations were located in the centromere regions. As a result, bridges were not formed and a large number of fragments were observed. On the other hand, chemicals may consistently produce chromosomal aberrations, but at the same time be ineffectual as clastogens. For example, pesticides which interfere with the spindle mechanism and thus induce C-mitosis generally possess only a very mild clastogenic effect.

Sister Chromatid Exchange

Sister chromatid exchange (SCE) involves a symmetrical exchange at one locus between sister chromatids. To my knowledge, the herbicide maleic hydrazide is the only pesticide that has been tested for the induction of SCE and it failed to induce SCE (*59*). Maleic hydrazide is an anomalous chemical, since it is a potent inducer of chromosome aberrations in plant cells (*60*) but it has never been reported to cause chromosome damage in mammalian cells.

Sensitivity

For a given class of chromosome aberrations, it has been shown that species vary in their sensitivity to pesticide treatment. For example, *Tradescantia* is less susceptible to chromosome breakage following pesticide treatment than *Vicia faba* (*44*). Barley is also less sensitive than *Vicia faba* (*61*). The susceptibility of a species to chromosome breakage has been shown to be related to level of ploidy, life-form and nuclear volume (*62*).

Use of Plants as a First-Tier Bioassay System

The question has been raised as to the relevance to human populations of data on chemically induced chromosome aberrations in plants (*63*). Furthermore, among the various test systems which have been recommended by a committee of the Environmental Mutagen Society, Committee 17 (*64*), no plant testing system has been included. However, a number of studies which have been carried out on pesticides indicate that there is an excellent correlation between chromosome abnormalities found in root-tip systems and those found in mammalian cell systems (Table 2). There is also a good correlation with mutagenic activity. It is true that the type of chromosome aberration induced by a specific chemical may not be the same in plant cells as in animal cells (*63*), but if a particular chemical will induce chromosome aberrations in one group, generally it will do so in the other as well. Fur-

Table 2. Comparison between chromosome-breaking and mutagenic effects of pesticides in plant and animals materials.

Compound	Chromosome aberrations: Plant root tips	Ref.	Chromosome aberrations: Mammalian cells in culture	Ref.	Mutagenic effect	Ref.
Apholate	+	(65)	+	(66)	?	
Atrazine	+	(31)	+	(67)	+	(68)
2,4-D	+	(52)	+	(69)	±	(58)
DDT	+	(70)	+	(71)	+	(72)
Dichlorvos	+	(73)	+	(74)	+	(75)
Dieldrin	+	(76)	+	(77)	±	(78)
Ethylene dibromide	−	(79)	−	(79)	+	(80)
Griseofulvin	+	(81)	+	(82)	−	(83)
Hempa	−	(65)	−	(84)	+	(85)
Heptachlor	+	(86)	+	(87)	+	(87)
Maleic hydrazide	+	(60)	−		+	(60)
Mercury compounds	+	(15)	+	(16)	?	
Phosphamidon	+	(50)	+	(88)	?	
2,4,5-T	+	(89)	+	(90)	±	(78)
Tepa	+	(65)	+	(91)	+	(85)

thermore, it has been shown that exactly the same morphologic "C-mitotic" picture occurs in plant and in animal tissue (*92*). This has been shown to be true for several mercurial compounds (*15, 16*) and griseofulvin (*81*), and possibly others. Thus, it is justified to assume that compounds which have a C-mitotic effect in plant tissue will induce the same effect in animal tissue.

Several higher plants provide unique and valuable systems for detecting and analyzing the effects of chemical mutagens (*3*). Such plants include maize (*Zea mays*), barley (*Hordeum vulgare*), tomato (*Lycopersicon*), mouse-ear cress (*Arabidopsis thaliana*), soybean (*Glycine max*), broad bean (*Vicia faba*), spiderwort (*Tradescantia*), onion (*Allium cepa*), Hawk's beard (*Crepis capillaris*), lily (*Lilium*), pea (*Pisum sativum*), and tobacco (*Nicotiana tabacum*). As a group, these plants offer systems for the analysis of almost all known genic and chromosomal aberrations which have been induced in eukaryotes by chemical or physical mutagens.

Some of the advantages in utilizing plant systems have been reviewed by previous authors (*3, 93*): (1) the chromosome organization of plants is similar to that of humans; (2) many plants are easy to grow; (3) some have short generation time; (4) the cost, handling, and space requirements are relatively small; (5) the cost and time of training technicians to handle a variety of end points following mutagen treatment is relatively small; (6) mutagenic effects can be studied under a wide range of environmental conditions such as large differences in pH, water content, temperature, and metabolic rates; (7) most of the plant systems have been in use for many years and are reliable systems which have been adapted for newer techniques such as chromosome banding and sister-chromatid exchange studies.

Perhaps the most serious disadvantage of a plant system for the detection of genetic risks to man is the lack of similarity between vegetative and mammalian metabolism. Nevertheless, the positive correlation which has been noted between aberrations induced by the same chemical in plant root-tip cells and in cultured mammalian cells indicates that a plant root-tip system must be recognized as an appropriate first-tier assay system.

Numerous studies have demonstrated that plant chromosomes are sensitive indicators to environmental pollutants. In this paper pesticides have been used to illustrate the potential of plant systems as monitors of chromosome aberrations. Pesticides are a diverse and extensively used group of chemicals and they are known to induce a wide range of chromosome aberrations. It is evident that plant systems are simple, reliable, and inexpensive. The application of the results obtained from mutagenesis in plants to humans is just as valid as those from the diploid organism *Neurospora*, an accepted test organism. Higher plant systems appear to be excellent indicators of the cytotoxic, cytogenetic, and mutagenic effects of environmental chemicals; and, therefore, it is recommended that plant systems be accepted as a first-tier assay system for the detection of possible genetic damage resulting from the use of environmental chemicals.

Financial assistance from the National Research Council of Canada is gratefully acknowledged. I thank Alina E. Russell for technical assistance.

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